



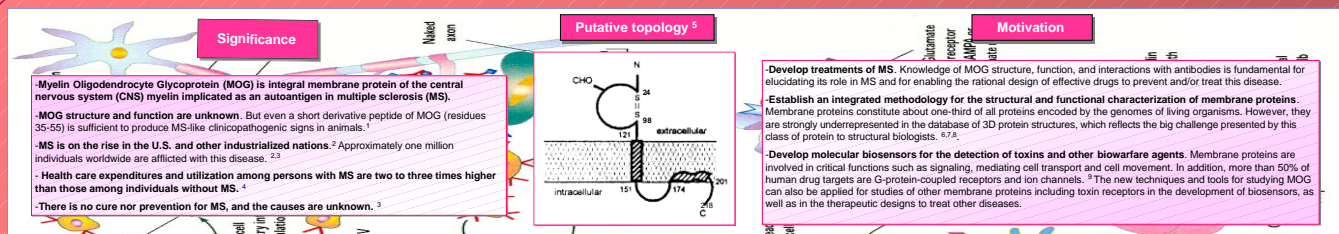
# Structural Determination of Myelin Oligodendrocyte Glycoprotein using Nuclear Magnetic Resonance Methods

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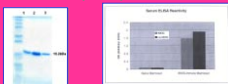
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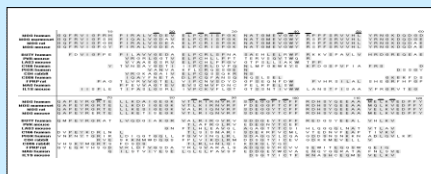
## The extracellular domain: rMOG(1-117)

- MOG(1-117) was successfully expressed and purified
- Reactivity tests: rMOG(1-117) was biologically active
- 56 amino acids in this sequence are hydrophobic
- Limited solubility in aqueous solutions



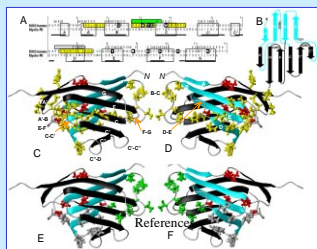
## Sequence alignments

Sequence alignments of the MOG extracellular domain in decreasing order of sequence homology for human, marmoset, rat, mouse, and the ten proteins with IgSF y-type fold obtained from the BLAST and PRODOM searches. Sequence identities and conserved amino acid residues are gray-shaded. IgSF y-type consensus residues are designated by heavy bars over the sequence for human MOG(1-120).



## Homology model of human MOG(2-120)

- Model is based on the analysis of immunoglobulin superfamily (IgSF) consensus residues and a sequence-structure alignment with the high resolution crystal structure of myelin PO.<sup>3</sup>
- The sequence alignment between human MOG (residues 1-120) and myelin PO (residues 1-119), indicating location of  $\beta$ -strands in these two sequences (open boxes), and in mouse MOG (heavy bars). The secondary structure (PHDsec) and solvent accessibility (PHDacc) predictions are shown on top of the human MOG sequence (E=extended, B=buried).
- The fine specificity of major T-cell (green), minor T-cell (gray), and B-cell (yellow) epitopes were found to be predominantly located on solvent-exposed regions in the model, and thus potentially accessible to antibody binding and/or proteolytic attack.

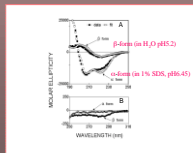
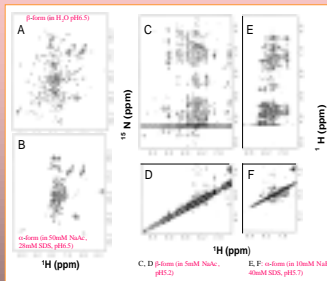


## Solvent-induced conformation changes in rMOG(1-117)

Solution state NMR and CD studies show that rMOG(1-117) adopts different tertiary structures depending on micro-environments.

### Key to uncovering the origins of MOG pathogenesis

- Characterization of rMOG(1-117) under various solvent conditions is important to better understand how in vivo environments, especially differences between the normal and disease states, can affect physical characteristics of this protein.



Solution additive	Relative mole $\alpha$ -helix
Dodecylphosphocholine (DPC)	9
Palmitoyllysophosphocholine (LPCP)	9
Palmitoyllysophosphatidic acid (LPPA)	15
Sodium lauryl sulfate (SDS)	12
45% Trifluoroethanol	20
No additive	9

## Acknowledgments

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## Conclusion

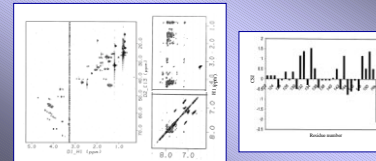
In addition to meeting enduring national needs in MS treatments, MOG is being used as a model system to validate an NMR-based methodology for a large variety of potential applications. The integrated methodology combines solution- and solid state NMR with traditional biochemical as well as computational techniques. LLNL is uniquely suitable to investigate such important classes of molecules, because of the laboratory's multidisciplinary approach to problem solving, wide-ranging capabilities, specialized research facilities, and extensive areas of expertise.

## The transmembrane domain: MOG(122-150)

- 100% hydrophobic sequence:  
NPGV LALIA LVPML LLQVS VGLVF LFLQ KKK

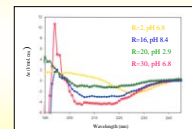
## MOG transmembrane (122-150) in DMSO

- DMSO is often chosen as the initial solvent for membrane proteins because it is non-polar, similar to the interior lipid environment of cell membranes.
- C $\alpha$  chemical shift index plot indicates the majority of residues in this segment assumes a random coil status in DMSO.



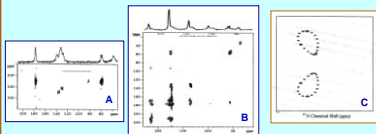
## MOG transmembrane in membrane-mimic environments

- DPC lipid:peptide ratio R=10: random coil structure
- R>10: helical conformations



## Explore the capabilities of high resolution SS-NMR

- Not limited by the requirements of having soluble or crystallizable proteins for structural determination by solution state NMR or by X-ray diffraction.
- Initial tests on the sample of phenylalanine: (A) <sup>13</sup>C-<sup>13</sup>C scalar coupling mediated HMQC, and (B) <sup>13</sup>C-<sup>1</sup>H dipolar coupling mediated HSCOC.
- Implementation of 15N-1H Polarization Inversion Spin Exchange at the Magic Angle (PISEMA) experiment (C) provide orientation information for each residue in a peptide.
- Employment of these methods for ubiquitously labeled membrane proteins would allow for the direct measurement of internuclear distances in such systems and provide key structural insights.



## References

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